

This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims:

1-30. (Canceled)

31. (Currently Amended) A method for detecting the presence of anti-MHC anti-class I MHC antibodies in a sample, the method comprising the steps of:

providing a substrate;

obtaining a pool of functionally active, recombinantly produced, truncated individual soluble class I MHC trimolecular complexes that have been purified substantially away from other proteins, each complex present in the pool comprising the same truncated, individual class I MHC heavy chain molecule, the pool obtained by the steps of:

isolating mRNA from a source, wherein the mRNA encodes [an] a class I MHC heavy chain allele;

reverse transcribing the mRNA to obtain cDNA;

identifying an individual class I MHC heavy chain allele in the cDNA;

PCR amplifying the individual class I MHC heavy chain allele in a locus-specific manner to produce a PCR product having the coding regions encoding cytoplasmic and transmembrane domains of the individual class I MHC heavy chain allele removed such that the PCR product encodes a truncated, soluble form of the individual class I MHC heavy chain molecule;

cloning the PCR product into a mammalian expression vector, thereby forming a construct that encodes the individual soluble class I MHC heavy chain molecule;

transfected a mammalian cell line with the construct to provide a mammalian cell line expressing a construct that encodes a recombinant, individual soluble class I MHC heavy chain molecule, wherein the mammalian cell line expresses beta-2-microglobulin and is able to naturally process proteins into peptide ligands for loading into antigen binding grooves of class I MHC molecules, and wherein the mammalian cell line expresses multiple surface-bound native Class class I endogenous MHC molecules; culturing the mammalian cell line under conditions which allow for expression of the recombinant individual soluble class I MHC heavy chain molecule from the construct, such conditions also allowing for endogenous loading of a peptide ligand into the antigen binding groove of each individual soluble class I MHC heavy chain molecule, and such conditions also allowing for noncovalent association of beta-2-microglobulin produced by the mammalian cell line with the individual soluble class I MHC heavy chain molecule and endogenously loaded, naturally produced peptide ligand to form the individual soluble class I MHC trimolecular complexes prior to secretion of the individual soluble class I MHC trimolecular complexes from the cell, and wherein each trimolecular complex of the pool of functionally active, recombinantly produced, truncated individual soluble class I MHC trimolecular complexes has the same recombinant, soluble class I MHC heavy chain allele; and

harvesting the soluble class I MHC complexes from the culture while retaining the mammalian cell line in culture for production of additional soluble class I MHC complexes; and

purifying the individual, soluble class I MHC trimolecular complexes substantially away from other proteins, wherein the individual

soluble class I MHC trimolecular complexes maintain the physical, functional and antigenic integrity of the native class I MHC trimolecular complex;

linking at least one soluble class I MHC trimolecular complex from the pool of functionally active, recombinantly produced, truncated individual soluble class I MHC trimolecular complexes to a substrate, wherein the at least one soluble class I MHC trimolecular complex is directly or indirectly linked to the substrate, and wherein the at least one soluble class I MHC trimolecular complex linked to the substrate retains the physical, functional and antigenic integrity of the native class I MHC trimolecular complex;

providing a sample;

reacting the sample with the substrate having the at least one class I MHC trimolecular complex linked thereto;

washing the substrate to remove unbound portions of the sample;

reacting the substrate having the at least one class I MHC trimolecular complex linked thereto with means for detecting anti-MHC anti-class I MHC antibodies; and

determining that anti-MHC anti-class I MHC antibodies specific for the individual class I MHC molecule are present in the sample if the means for detecting anti-MHC anti-class I MHC antibodies is positive.

32. (Previously Presented) The method of claim 31 wherein, in the step of providing a substrate, the substrate is a solid support.

33. (Previously Presented) The method of claim 32 wherein the solid support is selected from the group consisting of a well, a bead, a membrane, an ELISA plate, and a matrix.

34. (Previously Presented) The method of claim 33 wherein the bead is selected from the group consisting of a flow cytometry bead, and a magnetic bead, and wherein the membrane is selected from the group consisting of a nitrocellulose membrane, a PVDF membrane, a nylon membrane, and acetate derivative.

35. (Currently Amended) The method of claim 31 wherein, in the step of linking a soluble class I MHC trimolecular complex to a substrate, the soluble class I MHC trimolecular complex is indirectly attached to the substrate via an anchoring moiety.

36. (Currently Amended) The method of claim 35 wherein the anchoring moiety comprises an antibody to the functionally active, individual soluble class I MHC trimolecular complex.

37. (Previously Presented) The method of claim 36 wherein the antibody is selected from the group consisting of W6/32, anti-beta 2m, pan-Class I or allele-specific antibodies and combinations thereof.

38. (Currently Withdrawn - Amended) The method of claim 35 wherein the anchoring moiety comprises a tail or tag attached to the functionally active, individual soluble class I MHC trimolecular complex, and the substrate is further defined as comprising an affinity reagent to which the tail or tag binds.

39. (Currently Withdrawn) The method of claim 38 wherein the tail or tag is a histidine tag, and the affinity reagent is selected from the group consisting of nickel, copper and combinations thereof.

40. (Currently Withdrawn) The method of claim 38 wherein the tail or tag is a biotinylation signal peptide, and the affinity reagent is avidin or streptavidin.

41. (Currently Withdrawn) The method of claim 38 wherein the tail or tag is a VLDLr or FLAG tail, and the affinity reagent is an antibody that recognizes the VLDLr or FLAG tail.

42-44. (Canceled)

45. (Previously Presented) The method of claim 31 wherein, in the step of isolating mRNA from a source, the source is selected from the group consisting of mammalian DNA and an immortalized cell line.

46. (Previously Presented) The method of claim 31 wherein, in the step of cloning the PCR product into a mammalian expression vector, the mammalian expression vector contains a promoter that facilitates increased expression of the truncated PCR product.

47. (Canceled)

48. (Currently Amended) The method of claim 31 wherein, in the step of PCR amplifying the individual class I MHC heavy chain allele, a primer utilized in the PCR amplification includes a sequence encoding a tail such that the soluble class I MHC heavy chain molecule encoded by the truncated PCR product contains a tail attached thereto that facilitates in purification of the soluble class I MHC trimolecular complexes produced there from or facilitates in direct binding of the soluble class I MHC trimolecular complexes to the substrate.

49. (Currently Amended) The method of claim 31 wherein, in the step of PCR amplifying the individual class I MHC heavy chain allele, a 3' primer utilized in the PCR amplification includes a stop codon incorporated therein.

50. (Currently Amended) The method of claim 31 wherein, in the step of purifying the individual, soluble class I MHC trimolecular complexes substantially away from other proteins, the functionally active, individual soluble class I MHC trimolecular complexes are purified by affinity chromatography and fractionation.

51. (Previously Presented) The method of claim 50 wherein the affinity chromatography utilizes a reagent selected from the group consisting of W6/32 antibodies, anti-b2m antibodies, pan-Class I antibodies or allele-specific antibodies, and combinations thereof.

52-59. (Canceled)

60. (Previously Presented) The method of claim 31 wherein, in the step of providing a sample, the sample is selected from the group consisting of serum, tissue, blood, cerebrospinal fluid, tears, saliva, lymph, dialysis fluid, organ or tissue culture derived fluids, fluids extracted from physiological tissues, and combinations thereof.

61. (Currently Amended) The method of claim 31 wherein, in the step of reacting the substrate having the class I MHC trimolecular complex linked thereto with means for detecting anti-MHC anti-class I MHC antibodies, the means for detecting anti-MHC anti-class I MHC antibodies is a labeled anti-human antibody recognizing human IgG, IgM or IgA antibodies.

62-92. (Canceled)